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Selective and indirect modulation of human multipotential and erythroid hematopoietic progenitor cell proliferation by recombinant human activin and inhibin

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ABSTRACT **Activin and inhibin are biomolecules that, respectively, enhance and suppress the release of follicle-stimulating hormone from pituitary cells *in vitro*. Purified recombinant human (rhu) activin A and inhibin A were assessed for their effects on colony formation *in vitro* by human multipotential (CFU-GEMM), erythroid (BFU-E), and granulocyte–macrophage (CFU-GM) progenitor cells. It was found that (*i*) rhu-activin A enhances colony formation by normal bone marrow erythroid and multipotential progenitor cells; (*ii*) purified rhu-inhibin A decreases activin, but not rhu-interleukin 3, rhu-granulocyte–macrophage colony-stimulating factor, or rhu-interleukin 4, enhancement of erythropoietin-stimulated colony formation by erythroid and multipotential progenitor cells; (*iii*) modulatory actions of rhu-activin and rhu-inhibin are mediated through monocytes and T lymphocytes within the marrow; (*iv*) actions are apparent in the absence or presence of serum; and (*v*) rhu-activin and rhu-inhibin have no effect on colony formation by granulocyte–macrophage progenitor cells. This defines an indirect mode of action and a specificity for activin and inhibin on multipotential and erythroid progenitor cells.**

Blood cells are derived from a small population of morphologically nonrecognizable cells in the bone marrow termed hematopoietic stem and progenitor cells (1, 2). Myeloid blood cell production is regulated by networks of cell interactions involving production and release from accessory cells of molecules such as the hematopoietic colony-stimulating factors (CSFs): multi-CSF [also termed interleukin 3 (IL-3)], granulocyte–macrophage (GM)-CSF, granulocyte (G)-CSF, macrophage-CSF, as well as erythropoietin (Epo), and interleukins 1–6 (3–5, 40). Activin and inhibin, which, respectively, enhance and suppress release of follicle-stimulating hormone from pituitary cells *in vitro* (6–9), have been shown to influence erythroid cells *in vitro* (10–12). Natural porcine activin enhanced colony formation by Epo-stimulated human bone marrow erythroid colony-forming units (CFUs) (12) [considered to be a precursor cell equivalent to the proerythroblast, which is derived from the erythroid progenitor cell, termed erythroid burst-forming units (BFU-E) (1)]. Natural ovine inhibin had suppressive effects on erythroid CFUs (12). Human activin and inhibin genes have been cloned (13) and subsequently the recombinant proteins have been expressed and purified. Inhibin molecules are dimers that contain a common α chain and a βA or βB chain; activins are homodimers or heterodimers of just the β chains (13). The present investigation demonstrates an action for purified recombinant human (rhu) activin A and inhibit A *in vitro* on human bone marrow erythroid (BFU-E) and multipotential [granulocyte, erythrocyte, macrophage, and megakaryocyte CFU (CFU-GEMM)] progenitor cells, but not on granulocyte–macrophage [granulocyte–macrophage CFU (CFU-GM)] progenitor cells, which is mediated through marrow T lymphocytes and monocytes.

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MATERIALS AND METHODS

Activin and Inhibin. Rhu-activin A was produced by a mammalian cell line stably transfected with an expression plasmid containing cDNA encoding the βA subunit of human inhibin. The protein was isolated from conditioned culture medium by gel filtration on Sephacryl S-200 columns, cationic-exchange chromatography on DEAE-Sepharose columns, and reversed-phase HPLC on Vydac C_8 columns essentially as described by others (14). The amino acid composition of the recombinant protein is the same as that predicted from the coding region of the nucleotide sequence. Amino-terminal sequence analysis of the first 14 residues yields a single sequence (Gly-Leu-Glu-Xaa-Asp-Gly-Lys-Val-Asn-Ile-Xaa-Xaa-Lys-Lys) that corresponds to native activin A (6). Biological activity was tested in a rat pituitary cell bioassay (9). The purified rhu-activin caused a 2-fold increase in follicle-stimulating hormone secretion with an ED_{50} of 2–3 ng/ml, in agreement with that reported for native activin A (6, 8). Rhu-inhibin A was produced in mammalian cells transfected with expression vectors carrying inhibin α- and βA chain coding DNA sequences. Expression medium was concentrated and inhibin A was purified by gel filtration, cation-exchange chromatography, and reversed-phase HPLC as described for activin. Amino-terminal sequence analysis corresponded to the predicted human α- and βA structures. In the *in vitro* pituitary cell bioassay (9), the maximum degree of inhibition for the 2-day assay was 60–65% compared to untreated controls. The approximate half-maximal concentration of inhibition was 2–3 ng/ml.

Bone Marrow Cells and Cell Culture. Bone marrow cells were obtained by aspiration from the posterior iliac crest of healthy volunteers who had given informed consent according to guidelines established by the Human Investigation Committee of the Indiana University School of Medicine. Bone marrow cells were separated into fractions of low-density cells, nonadherent low-density T-lymphocyte-depleted cells, nonadherent low-density cells, or low-density T-lymphocyte-depleted cells as described elsewhere (15) by using density-cut procedures, adherence to plastic dishes, erythrocyte-rosette depletion, or some combination of these procedures. The nonadherent fractions contained <3% non-

Abbreviations: BFU-E, erythroid burst-forming unit; CFU, colony-forming unit; CSF, colony-stimulating factor; Epo, erythropoietin; G, granulocyte; GEMM, granulocyte, erythroid cell, macrophage, and megakaryocyte; GM, granulocyte–macrophage; IL-3 and -4, interleukin 3 and 4, respectively; rhu, recombinant human.

specific esterase-positive cells while the T-depleted fractions contained ≤3% T lymphocytes, as determined by flow cytometry with monoclonal antibody Leu4 or Leu5b (Becton Dickinson Monoclonal Center). Low-density and nonadherent low-density fractions were plated at 10^5 cells per ml whereas nonadherent low-density T-lymphocyte-depleted and low-density T-lymphocyte-depleted fractions were plated at 5×10^4 cells per ml.

For the BFU-E and CFU-GEMM assay, bone marrow cells were plated in a Petri dish containing 1 ml of 1% methylcellulose culture medium with Iscove's modified Dulbecco's medium (GIBCO), 30% (vol/vol) fetal calf serum (Hyclone), 5×10^{-5} M 2-mercaptoethanol, 1 unit of Epo (Toyoba, New York), with or without 0.1 mM hemin (Eastman Kodak). Cells were incubated in a humidified chamber at 5% CO_2 and 5% O_2 tension for 14 days and four plates per point were scored in each experiment for erythroid and multipotential colonies as described elsewhere (15). Low (5%) O_2 tension was maintained with the use of an Oxyreducer (Reming Bioinstruments, Redfield, NY). IL-3, GM-CSF, and interleukin 4 (IL-4) were purified preparations of recombinant human materials (specific activities, $\geq 10^8$ units/mg) and were kind gifts from Steven Gillis (Immunex, Seattle, WA). The erythroid assay was also set up with rhu-Epo (Amgen Biologicals) under serum-free conditions with the following ingredients (Sigma): bovine serum albumin at 10 mg/ml, iron-saturated human transferrin at 300 μg/ml, cholesterol at 7.8 μg/ml, and calcium chloride at 280 μg/ml, as described elsewhere (16).

For the CFU-GM assay (41), 10^5 low-density normal human bone marrow cells were plated in 1 ml in a Petri dish with 0.3% agar (Difco) culture medium containing McCoy's 5A medium supplemented with additional essential and nonessential amino acids, glutamine, serine, asparagine, sodium pyruvate (GIBCO), and 10% (vol/vol) heat-inactivated (56°C for 30 min) fetal calf serum, in the absence of exogenously added growth factors (− CSF) or in the presence of growth factors (+ CSF) present in medium conditioned by the urinary bladder carcinoma cell line 5637, purified rhu-GM-CSF or rhu-G-CSF (specific activity, $\geq 10^8$ units/mg of protein; a kind gift from Peter Ralph, Cetus Corp.). Three plates were scored for each point. Colony (>40 cells per aggregate) and cluster formation (3–40 cells per aggregate) was checked after 7 and 14 days since these scoring days detect CFU-GM at different stages of maturity (17, 18).

Statistical Analysis. The results are expressed as the mean ± 1 SEM and the probability of significant differences between groups was determined with the use of Student's *t* test.

RESULTS

The effects of rhu-activin were evaluated on colony formation by BFU-E and CFU-GEMM in low-density normal human bone marrow cells (Table 1). Epo is required for colony formation by BFU-E and CFU-GEMM, and hemin enhances this colony formation (15). No erythroid or multipotential colonies formed in the presence of rhu-activin at 12.5–100 ng/ml when Epo was absent, with or without hemin (0.1 mM) (data not shown). In the presence of Epo, with or without hemin, rhu-activin enhanced formation of BFU-E and CFU-GEMM colonies with plateau numbers apparent with rhu-activin at 50 ng/ml (Table 1). In 10 experiments, rhu-activin at 50 ng/ml significantly ($P < 0.005$) enhanced BFU-E colony formation by 106 ± 21% (with control colony numbers ranging from 42 to 113 colonies) and enhanced CFU-GEMM colony formation by 597 ± 141% (with control colony numbers ranging from 0.3 to 2 colonies). Activin enhancement was similar to that seen with concentrations of rhu-IL-3, rhu-GM-CSF, and rhu-IL-4 that are maximal for these effects (Table 2). To determine if the effects of rhu-activin were mediated by prostaglandins, cultures were set up in the absence and presence of 1 μM indomethacin and in the absence and presence of hemin. The activin-enhancing effects on Epo-stimulated colony formation by BFU-E and CFU-GEMM were similar in the absence or presence of indomethacin (Table 1).

Rhu-inhibin was assessed on Epo-stimulated colony formation by BFU-E and CFU-GEMM in low-density normal human bone marrow cells in the absence of hemin and in the absence or presence of rhu-activin, rhu-IL-3, rhu-GM-CSF, or rhu-IL-4 (Table 2). Inhibin (50 ng/ml) significantly suppressed colony formation by BFU-E and CFU-GEMM in the presence of rhu-activin, but had little or no effect on colonies formed in the presence of Epo, Epo plus rhu-IL-3, rhu-GM-CSF, or rhu-IL-4. A dose-related suppression of colonies was seen with rhu-inhibin in the presence of Epo (1 unit/ml) and rhu-activin (50 ng/ml). Numbers of BFU-E colonies in the presence of control diluent, rhu-inhibin at 60, 40, 20, 10, and 5 ng/ml were, respectively, 138 ± 3, 26 ± 2, 42 ± 1, 54 ± 5, 73 ± 7, and 100 ± 7 colonies. Numbers of CFU-GEMM colonies were, respectively, 5.5 ± 1.0, 0.5 ± 0.3, 1.5 ± 0.5,

Table 1. Influence of purified rhu-activin on colony formation by normal human bone marrow erythroid (BFU-E) and multipotential (CFU-GEMM) progenitor cells

	BFU-E, no. of colonies			CFU-GEMM, no. of colonies		
	− hemin		+ hemin	− hemin	+ hemin	
	Effect of rhu-activin					
McCoy's medium (control)	44 ± 3	72 ± 5	62 ± 3	1.3 ± 0.3	0.5 ± 0.3	1.0 ± 0.4
Control diluent	48 ± 4	68 ± 4	67 ± 4	0.5 ± 0.5	0.3 ± 0.3	0.8 ± 0.5
Rhu-activin (100 ng/ml)	122 ± 5*	133 ± 3*		7.1 ± 0.3*	5.0 ± 0.7*	
Rhu-activin (50 ng/ml)	139 ± 16*	128 ± 2*	168 ± 8*	7.8 ± 2.1*	3.8 ± 0.3*	5.8 ± 1.0†
Rhu-activin (25 ng/ml)	89 ± 5*	85 ± 6†	108 ± 10*	3.5 ± 0.7*	1.8 ± 0.3*	3.0 ± 0.4†
Rhu-activin (12.5 ng/ml)	56 ± 1*	67 ± 8	65 ± 3	1.3 ± 0.3	0.5 ± 0.3	1.3 ± 0.3
	Effect of indomethacin and rhu-activin					
Control medium		26 ± 1	43 ± 4		1.0 ± 0.3	
Control medium plus 1 μM indomethacin		28 ± 3	45 ± 3		1.3 ± 0.5	
Rhu-activin (50 ng/ml)		48 ± 3*	95 ± 5*		4.0 ± 0.7*	
Rhu-activin (50 ng/ml) plus 1 μM indomethacin		46 ± 3*	101 ± 4*		4.0 ± 0.5*	

Low-density (<1.077 g/cm³) normal human bone marrow cells were plated at 10^5 cells per ml with or without 0.1 mM hemin (Eastman Kodak) in the presence of either McCoy's medium, control diluent (containing the amount of trifluoroacetic acid/propanol in which the activin was diluted), or purified rhu-activin. Each column contains data from independent experiments.
*Significant change from McCoy's medium, *P* at least <0.005.
†Significant change from McCoy's medium, $P < 0.05$.

Table 2. Influence of bone marrow adherent cells and T lymphocytes on the effects of rhu-inhibin on colony formation by BFU-E and CFU-GEMM stimulated with purified preparations of rhu-activin, IL-3, GM-CSF, or IL-4

	Colonies, no.							
	LD cells		NALDT⁻ cells		NALD cells		LDT⁻ cells	
	Control medium	Inhibin	Control medium	Inhibin	Control medium	Inhibin	Control medium	Inhibin
BFU-E								
McCoy's medium	80 ± 6	73 ± 4	50 ± 1	52 ± 5	64 ± 3	63 ± 5	90 ± 5	87 ± 4
Rhu-activin (50 ng)	141 ± 8*	55 ± 5†	54 ± 2	56 ± 4	67 ± 5	47 ± 3	88 ± 2	88 ± 6
Rhu-IL-3 (200 units)	136 ± 10*	131 ± 11	117 ± 5*	122 ± 4	117 ± 4*	129 ± 6	150 ± 8*	151 ± 12
Rhu-GM-CSF (200 units)	115 ± 3*	112 ± 9	110 ± 3*	107 ± 10	92 ± 6*	81 ± 3	127 ± 5*	129 ± 8
Rhu-IL-4 (1000 units)	144 ± 7*	136 ± 5	131 ± 8*	123 ± 9	117 ± 9*	112 ± 3	143 ± 9*	144 ± 7
CFU-GEMM								
McCoy's medium	0.8 ± 0.3	0.8 ± 0.3	0	0	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3
Rhu-activin (50 ng)	5.0 ± 1.1*	0.8 ± 0.5†	0	0	0.5 ± 0.3	0.5 ± 0.3	0.8 ± 0.3	0.8 ± 0.3
Rhu-IL-3 (200 units)	8.0 ± 1.0*	7.0 ± 0.4	10.0 ± 1.0*	11.0 ± 1.0	8.0 ± 0.7*	8.0 ± 0.9	15.0 ± 0.6*	15.0 ± 0.7
Rhu-GM-CSF (200 units)	4.8 ± 0.3*	5.3 ± 0.5	4.8 ± 0.5*	3.8 ± 0.5	4.0 ± 0.7*	4.8 ± 0.6	7.0 ± 1.4*	7.0 ± 0.4
Rhu-IL-4 (1000 units)	3.0 ± 0.6*	3.0 ± 0.2	2.3 ± 0.3*	2.0 ± 0	2.0 ± 0.4*	1.5 ± 0.3	0.8 ± 0.3*	0.5 ± 0.3

Cultures were established in the absence of hemin and where indicated 50 ng of inhibin was added. The results have been confirmed in one other complete experiment by using these four fractions of separated cells and in three other experiments by using low-density (LD) cells. NALDT⁻, nonadherent low-density T-lymphocyte depleted; NALD, nonadherent low density; LDT⁻, low-density T-lymphocyte depleted.
*Significant increase in colony formation in the absence of inhibin compared to McCoy's medium control, P at least <0.005. Other numbers are not statistically different from McCoy's medium, $P > 0.05$.
†Significant decrease in colony formation by inhibin in the presence of McCoy's medium, activin, IL-3, GM-CSF, or IL-4 compared to these cultures in the absence of inhibin, $P < 0.005$. Other numbers are not statistically different, $P > 0$.

1.5 ± 0.5, 2.5 ± 0.7, and 3.3 ± 0.9 colonies. To evaluate the specificity of inhibin activity, the effects of rhu-activin, rhu-IL-3, rhu-GM-CSF, and rhu-IL-4 were assessed alone and with rhu-inhibin on Epo-stimulated colony formation of BFU-E and CFU-GEMM in the absence or presence of monocytes, T lymphocytes, or both (Table 2). Removal of the monocytes, T lymphocytes, or both ablated the rhu-activin and rhu-inhibin, but not the rhu-IL-3, rhu-GM-CSF, or rhu-IL-4, effects.

To assess whether effects were mediated by serum components or unknown materials in the crude preparation of Epo, rhu-activin and rhu-IL-3 were assayed alone, in the presence of rhu-inhibin, in the absence and presence of defined culture ingredients (±fetal calf serum), and in the presence of purified rhu-Epo. Both rhu-activin and rhu-inhibin were active in the absence of serum (Table 3). Removal of monocytes and T lymphocytes ablated the effects of rhu-activin and rhu-inhibin under serum-free conditions (data not shown).

To further define specificity of action, rhu-activin and rhu-inhibin were tested for effects on colony formation by granulocyte-macrophage progenitor cells (CFU-GM) in low-density marrow cells plated in the absence and presence of various sources of CSF (Table 4). Colonies and clusters

Table 3. Comparative influence of purified rhu-inhibin on colony formation by normal human bone marrow BFU-E stimulated with rhu-Epo in the presence of rhu-activin or rhu-IL-3 under serum-containing or serum-free culture conditions

	Colonies (BFU-E), no.			
	+ serum		− serum	
	Control medium	+ inhibin	Control medium	+ inhibin
Experiment 1				
Control medium	37 ± 5	33 ± 8	10 ± 2	10 ± 4
Rhu-activin (50 ng)	92 ± 4*	38 ± 3†	41 ± 2*	15 ± 2†
Rhu-IL-3 (200 units)	109 ± 7*	103 ± 5	48 ± 4*	46 ± 7
Experiment 2				
Control medium	42 ± 5	37 ± 3	7 ± 2	9 ± 2
Rhu-activin (50 ng)	102 ± 2*	33 ± 4†	20 ± 5*	8 ± 2†
Rhu-IL-3 (200 units)	95 ± 4*	88 ± 4	30 ± 4*	27 ± 4

Low-density normal human bone marrow cells were plated at 10^5 cells per ml in the presence of 30% (vol/vol) fetal calf serum with 1 unit of purified rhu-Epo (Amgen Biologicals) in the absence of hemin or were plated under serum-free culture conditions with rhu-Epo as described herein and elsewhere (16). Inhibin (30 ng) was added as indicated.
*Significant increase in colony formation in the absence of inhibin compared to McCoy's medium, $P < 0.005$.
†Significant decrease in colony formation by inhibin in the presence of activin, $P < 0.005$. Values for inhibin plated in the presence of either control medium or IL-3 were not statistically different from those cultures plated in the absence of inhibin, $P > 0.05$.

Table 4. Purified rhu-activin and rhu-inhibin have no effect on colony formation by CFU-GM cells in the absence or presence of exogenously added CSFs

	Day 7 CFU-GM, no.				Day 14 CFU-GM, no.			
	− CSF		+ CSF		− CSF		+ CSF	
	Col.	Col. + clust.	Col.	Col. + clust.	Col.	Col. + clust.	Col.	Col. + clust.
Experiment 1								
McCoy's medium	0	162 ± 9	39 ± 2	105 ± 10	11 ± 3	54 ± 4	29 ± 2	55 ± 3
Rhu-activin (50 ng)	0	154 ± 11	43 ± 1	96 ± 3	11 ± 1	48 ± 1	27 ± 3	52 ± 5
Experiment 2								
McCoy's medium	0	271 ± 8	0	314 ± 11	19 ± 2	79 ± 4	36 ± 3	76 ± 5
Rhu-activin (50 ng)	0	260 ± 21	0	306 ± 11	19 ± 1	79 ± 7	35 ± 1	73 ± 1
Experiment 3								
McCoy's medium	21 ± 4	174 ± 3	88 ± 5	147 ± 7	49 ± 1	62 ± 4	64 ± 2	84 ± 4
Rhu-activin (100 ng)	21 ± 2	173 ± 6	90 ± 5	136 ± 8	47 ± 2	61 ± 5	60 ± 3	88 ± 5
Rhu-inhibin (60 ng)	20 ± 6	180 ± 5	82 ± 4	143 ± 7	51 ± 2	65 ± 4	63 ± 6	79 ± 7
Experiment 4a								
McCoy's medium	0	283 ± 7	46 ± 6	167 ± 9	8 ± 1	50 ± 5	37 ± 2	70 ± 4
Rhu-inhibin (20 ng)	0	287 ± 8	40 ± 3	158 ± 12	9 ± 1	52 ± 1	36 ± 2	66 ± 1
Experiment 4b								
McCoy's medium			22 ± 3	178 ± 14			32 ± 4	65 ± 3
Rhu-inhibin (20 ng)			21 ± 3	184 ± 8			34 ± 6	71 ± 9
Experiment 4c								
McCoy's medium			24 ± 2	118 ± 5			19 ± 3	49 ± 5
Rhu-inhibin (20 ng)			25 ± 3	112 ± 5			20 ± 2	48 ± 2

Low-density normal human bone marrow cells were plated at 10^5 cells per ml in the absence of exogenously added growth factors (− CSF) or in the presence of growth factors (+ CSF) present in medium conditioned by the urinary bladder carcinoma cell line 5637 or by purified rhu-GM-CSF or by rhu-G-CSF. The source of added CSF for experiments 1, 3, and 4a was 10% (vol/vol) 5637 conditioned medium. Experiment 2 used 1% 5637 conditioned medium. Experiments 4b and 4c used, respectively, 200 units of rhu-GM-CSF or 200 units of rhu-G-CSF as an exogenous source of CSF. All parts of experiment 4 used the same marrow sample, whereas experiments 1–3 used different marrow samples. Col., >40 cells per aggregate; clust., 3–40 cells per aggregate.

formed in the absence of exogenously added CSF result from endogenous production/release of CSFs from accessory cells present in the low-density marrow cells (1). Neither rhu-activin nor rhu-inhibin had significant effects on colony or cluster formation in the absence or the presence of CSFs. Similar results were seen in three other experiments whether cells were plated in the presence or absence of fetal calf serum with low-density or nonadherent low-density T-lymphocyte-depleted bone marrow (data not shown).

DISCUSSION

These studies define a selective and indirect mode of action for the respective enhancing and suppressing effects of rhu-activin A and rhu-inhibin A on colony formation *in vitro* by human bone marrow BFU-E and CFU-GEMM. These indirect effects are in contrast to the direct effects noted for activin and inhibin on hemoglobin accumulation in the established murine Friend and human K562 cell lines (10–12). Although the mechanisms of action of these two molecules on normal cells are not yet known, we speculate that rhu-activin and rhu-inhibin may respectively induce and suppress release of growth factors from accessory cells. Released factors alone or in combination with rhu-activin or rhu-inhibin might act directly on BFU-E and CFU-GEMM. Lack of effect of rhu-activin and rhu-inhibin on CFU-GM is intriguing because it suggests possibly the induced release of factors that are specific for BFU-E and CFU-GEMM. The activin-induced production of growth factors for CFU-GM, BFU-E, and CFU-GEMM plus inhibitors for CFU-GM only is highly unlikely based on the studies in Table 4 and the studies with indomethacin reported in the text. IL-3 (19–21), GM-CSF (16, 21–23), IL-4 (21), G-CSF (16, 21, 24), and prostaglandin E (15) enhance colony formation by BFU-E, CFU-GEMM, or both, but also influence colony formation by CFU-GM. Interferon γ (25–27), tumor necrosis factors α and β (28–30), interleukin 1 (31–33), and IL-4 (21) induce release of growth factors from accessory cells, but these stimulate CFU-GM, BFU-E, and CFU-GEMM. A purified lymphocyte membrane-derived human growth factor enhances colony formation by BFU-E, but not by CFU-GEMM, CFU-GM, or megakaryocyte progenitors (34). An erythroid-potentiating activity (35) is a human tissue inhibitor of metalloproteinases (42). Inhibin has DNA sequence homology with transforming growth factor β (36), but transforming growth factor β while suppressing growth of erythroid colony-forming units, BFU-E, and CFU-GEMM enhances or suppresses growth of CFU-GM (37, 38). Since Epo was used at maximal concentrations, it is not likely that rhu-activin or rhu-inhibin were influencing release of Epo. This suggests that rhu-activin and rhu-inhibin may influence production or action of molecules such as the erythroid potentiating activity or other molecules not referred to above. The *in vivo* relevance of our results remains to be determined, but our findings suggest an added degree of specificity to the regulation of myeloid blood cell production. In this context, it may be significant that mRNA expression of the βA subunit of inhibin is found predominantly in placenta and bone marrow (39). α-Chain expression has not been detected in bone marrow suggesting that the activity of β dimers (activins) may predominate over α–β dimers (inhibins) in that organ (39), thus allowing for activin stimulation of certain elements of myelopoiesis.

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